

Hepatic Effects of Phthalate Esters

by Prahlad K. Seth*

Di(2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer and microchemical environmental pollutant, produces subtle changes in hepatic function as judged by increase in liver weight and morphological and biochemical alterations. It can modify the biological response of drugs and other xenobiotics. Such interactions appear to occur at the pharmacokinetic phase, as DEHP was found to alter the activity of microsomal drug-metabolizing enzymes and ethanol metabolism. DEHP produced a time- and route-dependent effect on the hepatic cytochrome P-450 contents and activity of aminopyrine *N*-demethylase, aniline hydroxylase, alcohol dehydrogenase and high and low K_m aldehyde dehydrogenases when given orally or intraperitoneally. Under *in vitro* conditions, DEHP produced no effect on the activity of aminopyrine *N*-demethylase or aniline hydroxylase, while mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) significantly inhibited their activity at concentrations ranging from 2.5 to 15.0 mM. Activity of aminopyrine *N*-demethylase and aniline hydroxylase was also inhibited by dimethyl phthalate (DMP) and dibutyl phthalate (DBP) after a single oral administration. In view of the possibility of the human exposure to phthalates and other xenobiotics simultaneously, these observations are of great significance.

Introduction

Phthalic acid esters (PAEs) are commonly used as plasticizers to impart flexibility to plastics, particularly poly(vinyl chloride) (PVC) polymers, which have a wide variety of biomedical and other uses. Di(2-ethylhexyl) phthalate (DEHP) and other PAEs are known to leach out from finished PVC products into blood, physiological fluids, commercial solvents and food materials (1, 2). Entry of these plasticizers into the biological system during transfusions (blood or physiological fluids) or hemodialysis or through food chain and their ubiquitous presence in environment has led to numerous studies on their toxicology. In spite of their low order of toxicity, DEHP and other phthalates have been shown to exert hepatotoxic, cytotoxic, teratogenic and mutagenic effects. Certain renal, pulmonary and reproductive dysfunctions have also been reported on exposure to these plasticizers in aquatic invertebrates and mammalian species (1-3). Liver and testis appear to be the main target organs in phthalate toxicity.

It is well established that the biological response of xenobiotics can be altered significantly due to their interactions with variables in external and

internal environmental and pharmacological agents. Unfortunately, this aspect of phthalate toxicity has not been studied in detail, though such information will be of great significance in assessing the toxicogenic potential of PAEs because of their ubiquity and hepatotoxic nature. This paper therefore reviews some of the hepatic effects of PAEs, with particular emphasis on their interaction with drugs and other xenobiotics.

Effect of Phthalates on Weight, Morphology and Chemical Constituents of Liver

Several investigators have studied the hepatotoxic potential of PAEs in a variety of animal species. Acute and chronic administration of DEHP caused variable effects on liver, depending upon the species of the animal (4). In general, phthalates after oral or intraperitoneal administration caused enlargement of the liver (5-9), which declined to or below normal weight on prolonged or discontinued exposure (10) (Table 1).

Histopathological examination of phthalate-induced enlarged livers showed fatty vacuolation and congestion followed by a cloudy swelling, excessive fatty degeneration (4, 11). Dilatation of smooth and rough endoplasmic reticulum, mitochondrial

*Industrial Toxicology Research Centre, P. O. Box 80, Lucknow-226 001, India.

Table 1. Effect of DEHP on weight, morphology and chemical constituents of liver.

Species	Route of administration	Principal findings	References
Rat	IP	Liver enlargement; no change in dry weight, moisture, pyruvic acid and lactic acid contents	(9)
Rat	Oral	Liver enlargement	(5,6,8)
Rat	Oral	Liver enlargement, which disappears on prolonged or discontinued exposure	(10)
Rat	Oral	Liver enlargement, proliferation of SER, increase in number of microbodies and swelling of mitochondria with shortening of cristae ^a	(7)
Rat	Oral	Cloudy swelling, necrosis and enlargement of liver cells	(11)
Mouse	Oral	Swollen mitochondria and increased SER ^a	(13)
Ferret	Oral	Increase in liver weight, enlarged parenchymal cells not distributed uniformly, lysosomal changes and dilatation of SER ^a	(12)
Monkey	IV	Liver necrosis, inflammatory cell filtration, subtle changes in BSP clearance rate	(14)

^aSER = Smooth endoplasmic reticulum.

Table 2. Effect of phthalates on energy and carbohydrate metabolism.

Species	Route of administration	Principal findings	References
Rat	Oral	Mitochondrial swelling and decreased SDH activity	(7)
Rat	Oral	Inhibition of glycolysis and decrease in glycogen content	(24)
Rat	IP	Inhibition of SDH, ATPase, diaphorase and cytochrome-c oxidase activities	(9,15,19)
Rat	(<i>In vitro</i>)	Mitochondrial swelling and stimulation of latent ATPase activity	(16)
Rat	(<i>In vitro</i>)	Inhibition of mitochondrial respiration, and SDH activity	(19)
Rat	(<i>In vitro</i>)	Inhibition of mitochondrial respiration	(17,18)
Rat	IP	Decreased blood glucose tolerance, glycogen, glycogenesis and glycogenolysis; inhibition of glucose-6-phosphatase, phosphorylase and glucose-6-phosphate dehydrogenase	(25)
Mouse	Oral	Decreased glycogen	(13)
Ferret	Oral	Absence of glycogen	(12)

swelling and increase in microbodies in rat liver have been reported on oral administration of DEHP for 21 days (7). Enlargement of cells, dilatation of smooth endoplasmic reticulum and changes in hepatic lysosomes of ferrets (12) and swollen mitochondria and increased smooth endoplasmic reticulum in liver of mice (13) receiving DEHP orally have also been reported.

Very little information is available on the hepatic effects of phthalates in primates. Jacobson (14) has reported liver necrosis and inflammatory cell infiltrations in rhesus monkeys receiving plasma-solubilized DEHP intravenously. Subtle changes in the clearance time of sulfobromophthalein were also observed (Table 1).

Effect of Phthalates on Energy and Carbohydrate Metabolism

Associated with liver enlargement, changes (increases or decreases) in many hepatic enzymes have been reported in phthalate-exposed animals (Table 2). A decrease in succinic dehydrogenase

(SDH) activity after oral or intraperitoneal administration of DEHP has been observed consistently (7, 15). Histochemical studies have revealed that loss of the enzyme occurs specifically in the periportal zones (7).

Mitochondria appear to be the target of phthalates. Adverse effects of phthalates on mitochondrial function have been reported by several investigators. *In vitro* studies have shown that several PAEs can produce significant inhibition of mitochondrial respiration (16-18). The studies of Ohyama (16) suggest that phthalates are electron and energy transport inhibitors, while the observations of Inouye et al. (10) indicate that phthalates can also cause uncoupling of oxidative phosphorylation. Phthalates were found to produce aberrations in mitochondrial function, e.g., di-n-butyl phthalate enhanced K⁺ efflux from isolated mitochondria, usually not produced by standard uncoupling agents (18). Sensitivity of various components of electron transport chain towards phthalates was indicated under *in vivo* conditions as well as by decreased activities of mitochondrial cytochrome-c oxidase, malic dehydrogenase and diaphorase in liver of DEHP-treated

rats (9). The inhibition of these mitochondrial enzymes also indicates an interaction of DEHP with mitochondrial membranes.

DEHP was also found to inhibit the activity of total and Mg^{+} -stimulated adenosine triphosphatase (ATPase) activity of rat liver (9, 19). Besides liver, the activity of SDH and ATPase was also inhibited in rat heart, lung, kidney (19) and gonads (20), indicating that suppression of energy-linked reactions may be a generalized effect of DEHP. *In vivo* effects of DEHP (9, 15, 19) were present 11 days after the final treatment with plasticizers. This suggests that enzymic alterations were perhaps not related to the physical presence of DEHP, as by this time all of the plasticizer would have been excreted (6, 21, 23).

Detailed studies have been conducted on the effects of DEHP on carbohydrate metabolism (Table 2). A decrease in glycogen in livers of mice (13), rats (19) and ferrets (12) receiving DEHP has been reported. Sakurai et al. (24) showed marked depression of glucose and glycogen in livers of rat receiving a diet containing 2% or 4% DEHP. Studies on the rates of incorporation of labeled pyruvate into blood glucose and liver glycogen and alteration in the levels of intermediates of carbohydrate metabolism indicated retardation of gluconeogenesis (24). Recent studies of Mushtaq et al. (25) using cold and labeled glucose have shown that DEHP inhibits both glycogenesis and glycogenolysis in rat liver. These authors have observed a significant decrease in the activity of glucose 6-phosphate dehydrogenase, phosphorylase and glucose 6-phosphatase and no change in the activity of fructose 1,6-diphosphatase and aldolase in livers of DEHP-exposed rats (25).

Effect of Phthalates on Lipid and Protein Metabolism

Since the detection of DEHP in association with triglycerides of beef mitochondria (26), its interaction with lipid metabolism has been a subject of active

investigation (Table 3). Stein et al. (27) showed an interaction between dietary fat and DEHP in lipid metabolism where presence of the phthalate in diet potentiated the growth-promoting effect of lipids in rats. Substantial reduction in serum cholesterol and proliferation of peroxisomes and elevation of catalase and carnitine acetyltransferase activity in livers of animals receiving DEHP via diet was reported by Reddy et al. (28). Induction of the fatty acyl-CoA oxidizing enzyme system located in peroxisomes by DEHP has been suggested by Osumi and Hashimoto (29), and inhibition of lipid synthesis in rats has been shown by Bell et al. (30, 31). Reduced serum glucose and cholesterol and elevated serum free fatty acid and ketone body have also been reported (26, 32, 33). The rats receiving 0.5% of DEHP in a normal protein diet showed accumulation of phospholipids, decrease in cholesterol and triglyceride contents in liver and a rise in levels of plasma fatty acids. Such observations suggest the utilization of lipids for the energy source. However, the significance of this altered state of energy metabolism is not understood at present (Table 3).

No significant studies have been conducted on the effects of phthalate on protein metabolism, though their effects on the other proximate principles, carbohydrate and fat have been studied in detail. An increase in the protein content in liver of DEHP-treated rats has been described by Yanagita et al. (32). Recently Pillai et al. (34) have shown that a DEHP-caused elevation in rat liver protein content is due to a decrease in protein breakdown (Table 3).

Interaction of Phthalates with Other Xenobiotics

Alterations in barbiturate sleeping time on exposure to phthalates have been reported by several investigators. A single intraperitoneal or intravenous injection of DEHP was found to increase the hexobarbital or pentobarbital sleeping time in rats or mice (6, 35-37). Repeated intraperitoneal ad-

Table 3. Effect of DEHP on lipid and protein metabolism.

Species	Route of administration	Principal findings	References
Rat	Oral	Inhibition of lipid biosynthesis	(30,31)
Rat	IP	Decrease in cholesterol content	(9)
Rat	Oral	Decrease in triglycerides and increase in phospholipids	(24)
Rat, mouse	Oral	Decrease in serum cholesterol; proliferation of peroxisomes; increase of catalase and carnitine acetyltransferase	(28)
Rat, mouse	Oral	Induction of enzymes of fatty acyl-CoA β -oxidation	(29)
Rat	Oral	Increase in mitochondrial and microsomal phospholipids; increase in protein content	(32,35)
Rat	Oral	Increase in protein content (decrease in protein degradation)	(34)

ministration of a number of phthalates caused significant and dose-dependent increases in pentobarbital sleeping time in mice (38), while repeated oral administration of DEHP resulted in reduced hexobarbital sleeping time in rats (6).

DEHP has also been shown to modify the biological response of parathion, an organophosphorus insecticide (39). Parathion exerts its toxic effect through its metabolite, paraxon, which is a potent inhibitor of acetylcholine esterase enzyme. A lower inhibitory activity of parathion in DEHP-treated rats suggested an effect of the phthalate on biotransformation of the pesticide to its active metabolite (39). Alterations in the biological response of carbon tetrachloride (40) and ethanol (41) have also been described.

The mechanism by which phthalates modify the biological response of the above-mentioned xenobiotics is not clear, although some investigators have suggested alterations in the biodistribution and biotransformation of the test xenobiotics or an increase in sensitivity of the central nervous system towards them in the presence of phthalates (6, 36-39). Estimation of concentration of barbiturates in blood during sleep and at awakening have shown that sleeping time alterations caused by DEHP were related to retarded disposition of the test drugs and not to the increased sensitivity of the central nervous system (36, 38). An increase in hepatic cytochrome P-450 content, alcohol dehydrogenase activity and persistent inhibition of aniline hydroxylase activity in livers of rats receiving DEHP for prolonged periods indicates its interaction with biotransformation mechanism.

In view of the significance of this aspect of phthalate toxicity and variable effects of phthalates on barbiturate and ethanol-induced sleeping time, the effect of DEHP on some enzymes involved in their biotransformation will be discussed in detail.

Effect of DEHP on Enzymes Involved in Biotransformation of Ethanol and Other Xenobiotics

The effect of DEHP on the activity of aminopyrine N-demethylase or aniline hydroxylase is shown in Figure 1. A single oral or intraperitoneal exposure to DEHP caused a significant dose-dependent decrease in aminopyrine N-demethylase and aniline hydroxylase activity and no change in cytochrome P-450 content (data not shown). Exposure to DEHP for 7 days produced a differential effect. Activity of these two microsomal enzymes was significantly increased upon oral administration but remained decreased upon intraperitoneal administration. Repeated oral administration also increased the levels

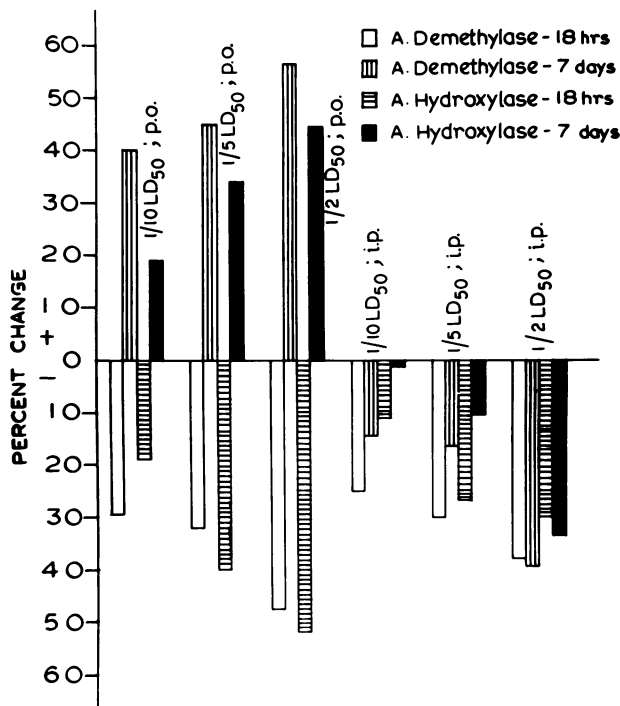


FIGURE 1. Effect of di(2-ethylhexyl) phthalate (DEHP) on aminopyrine N-demethylase (A. demethylase) and aniline hydroxylase (A. hydroxylase) activity of rat liver. Adult male albino rats were treated with 1/10, 1/5 and 1/2 of the LD₅₀ dose of DEHP orally (LD₅₀ = 26 g/kg) by gavage or intraperitoneally (LD₅₀ = 50 ml/kg) once or daily up to 7 days. These doses are known to cause teratogenic (42), pharmacologic (38) and biochemical (7-9, 15, 19, 20) effects.

of cytochrome P-450 which were not altered upon single oral administration (data not shown). Cytochrome P-450 levels were also not affected by repeated intraperitoneal administration of DEHP.

In order to see whether the alterations in activities of aminopyrine N-demethylase and aniline hydroxylase are caused by DEHP or its two active metabolites, their effects on these two enzymes were investigated *in vitro* at varying concentrations. DEHP had no effect on aminopyrine N-demethylase (Fig. 2) or aniline hydroxylase activity (Fig. 3), while mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) produced significant inhibition at concentrations ranging from 2.5 to 15mM.

The effects of DEHP on activity of alcohol dehydrogenase and high and low K_m aldehyde dehydrogenases are shown in Figures 4 and 5. A single oral administration of DEHP significantly inhibited the activity of alcohol dehydrogenase in a dose-dependent manner while intraperitoneal administration produced the effect only at the highest dose. No effect of DEHP was observed on the activity of high or low K_m aldehyde dehydrogenase after a single oral or intraperitoneal administration (Fig.

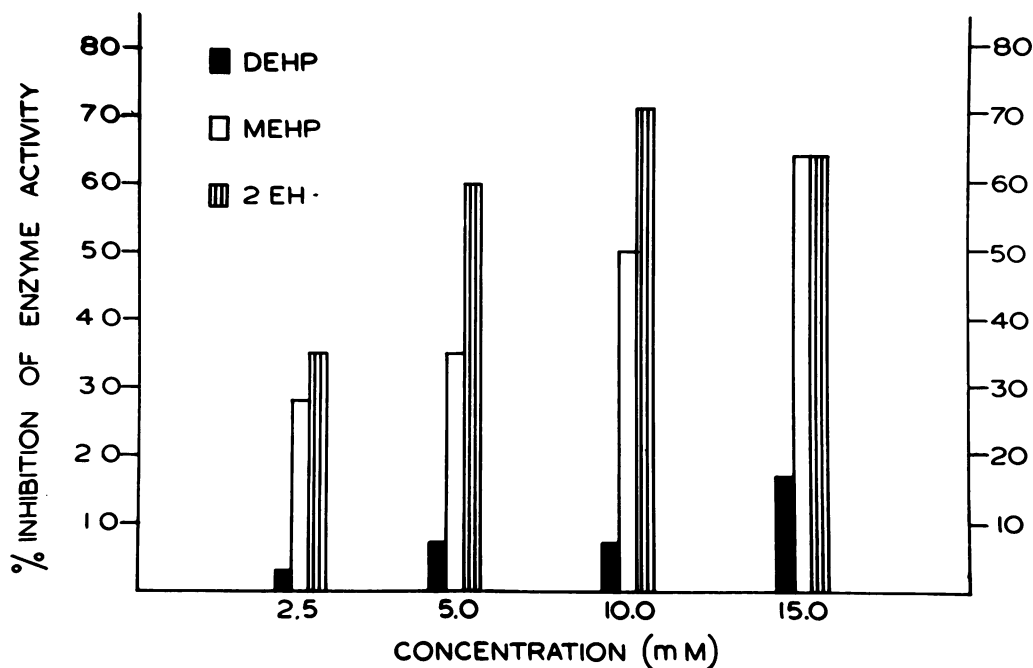


FIGURE 2. *In vitro* effect of di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) on the activity of aminopyrine *N*-demethylase of rat liver. These compounds were added directly into the incubation mixture and the effect was studied without any preincubation with enzyme preparation.

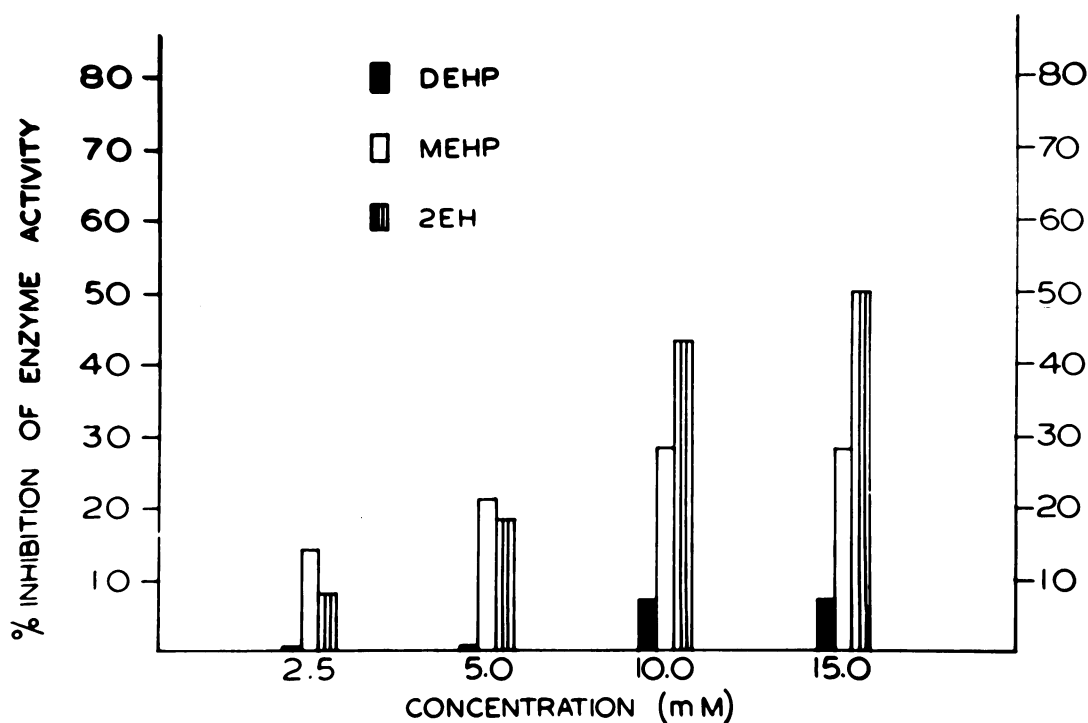


FIGURE 3. *In vitro* effect of di(2-ethylhexyl) phthalate (DEHP), mono(2-ethylhexyl) phthalate (MEHP) and 2-ethylhexanol (2-EH) on the rat liver aniline hydroxylase activity. Conditions of assay as described in Fig. 2.

4). The repeated oral administration of DEHP produced a different effect than that seen after a single administration of the phthalate. The activities of alcohol and aldehyde dehydrogenases were significantly increased. However, repeated intraperitoneal administration of DEHP caused no significant change in the activity of these enzymes (Fig. 5).

The above results suggest that DEHP produces a time- and route-dependent effect on the activities of aminopyrine *N*-demethylase, aniline hydroxylase, cytochrome P-450 content and alcohol dehydrogenase and high and low K_m aldehyde dehydrogenases. A similar time- and route-dependent effect of DEHP was seen on the pentobarbital (43) and ethanol (44) sleeping time in rats and mice, respectively. An increase in hexobarbital sleeping time after intraperitoneal or intravenous administration and decrease in the same after repeated oral administration have also been observed by Daniel and Bratt (6). However, no suitable explanation has been provided by these investigators. DEHP is hydrolyzed to MEHP at markedly different rates by intestine and liver lipases

(21, 45, 46) and metabolized to 2-EH. These two major metabolites of DEHP have been reported to mimic some of the biological effects of plasticizer (7, 16, 33). Thus, the above observed route-dependent effects of DEHP may perhaps be related to the amounts of MEHP and 2-EH formed in the body, as oral administration of the phthalate may result in higher concentrations of these metabolites.

A significant inhibition of aminopyrine *N*-demethylase and aniline hydroxylase by MEHP and 2-EH without any effect of DEHP on these enzymes under *in vitro* conditions suggests that these two metabolites are responsible for their *in vivo* effects. The DEHP-induced alterations in ethanol metabolism may also be related to MEHP and 2-EH, as these metabolites can be generated both by mitochondria and microsomes (45). Such observations are of great significance in view of a recent report on the metabolism of DEHP and accumulation of its metabolites in human blood and red cell concentrates stored in PVC blood bags (47).

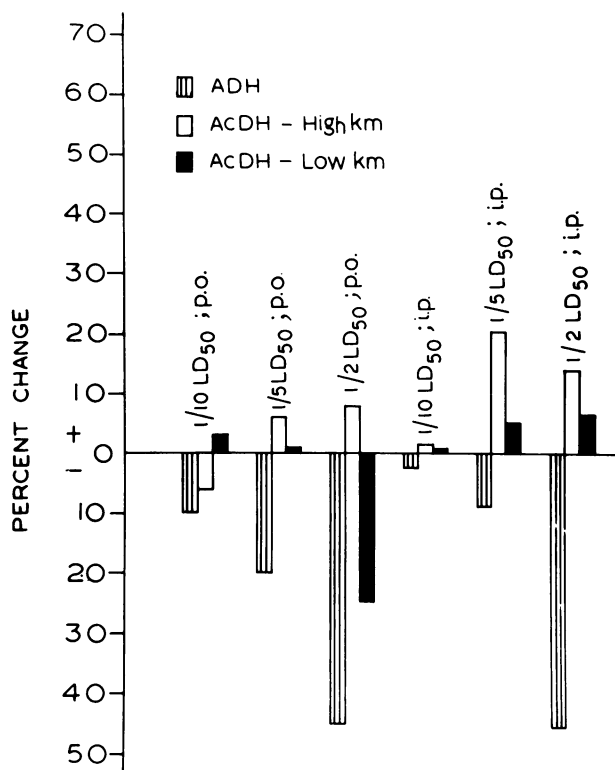


FIGURE 4. Effect of DEHP on alcohol dehydrogenase (ADH), high K_m aldehyde dehydrogenase (ACDH high K_m) and low K_m aldehyde dehydrogenase (ACDH low K_m). Adult male Swiss mice were treated with 1/10, 1/5 and 1/2 LD₅₀ dose orally (LD₅₀ = 40 ml/kg) by gavage, and enzyme activities were measured in liver 18 hr after the treatment.

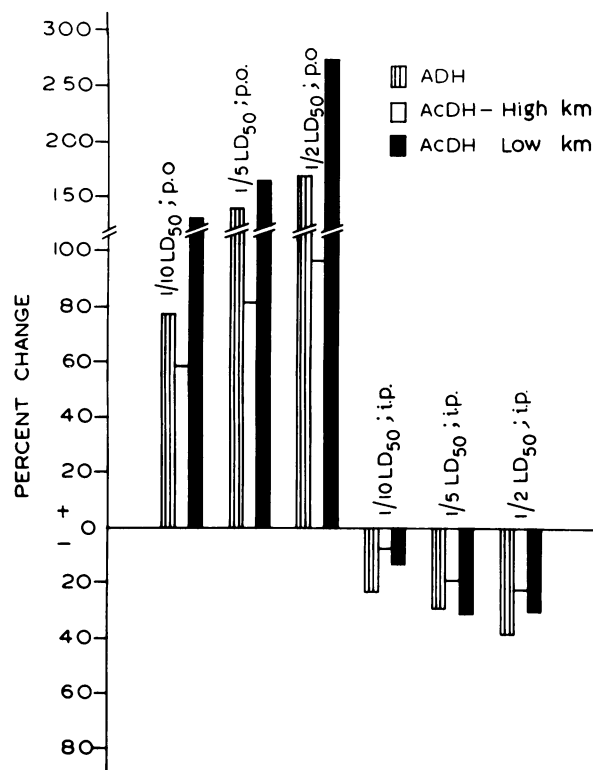


FIGURE 5. Effect of DEHP on alcohol dehydrogenase (ADH), high K_m aldehyde dehydrogenase (ACDH high K_m) and low K_m aldehyde dehydrogenase (ACDH low K_m) activity of mice liver. Adult male Swiss mice were given three different doses of DEHP as described in Fig. 5 daily for 7 days, and enzyme activities were measured 18 hr after the last treatment.

Effect of Dimethyl Phthalate (DMP) and Dibutyl Phthalate (DBP) on Drug-Metabolizing Enzymes

In order to compare the effects of DMP with DEHP on the activity of aminopyrine *N*-demethylase and aniline hydroxylase, male rats were exposed to these phthalates at a concentration corresponding to 1/10 of their LD₅₀ dose by intraperitoneal route. The activity of these enzymes was examined 18 hr after the administration of phthalates. All the three phthalates inhibited the activity of aminopyrine *N*-demethylase and aniline hydroxylase with DMP producing the maximum effect followed by DEHP and DBP (Fig. 6). The effect of DBP on aminopyrine *N*-demethylase was, however, not significant.

The above results show that besides DEHP, these two phthalates can also inhibit the activity of drug-metabolizing enzymes and hence can modify the biological response of certain xenobiotics. These phthalates failed to exhibit any structure-activity relationship in affecting the activity of aminopyrine *N*-demethylase and aniline hydroxylase. A similar observation on the lack of any role of molecular structure of phthalates in prolonging the pentobarbital sleeping time (38) or inhibiting the growth of nerve cell fibroblasts (48) has been reported earlier.

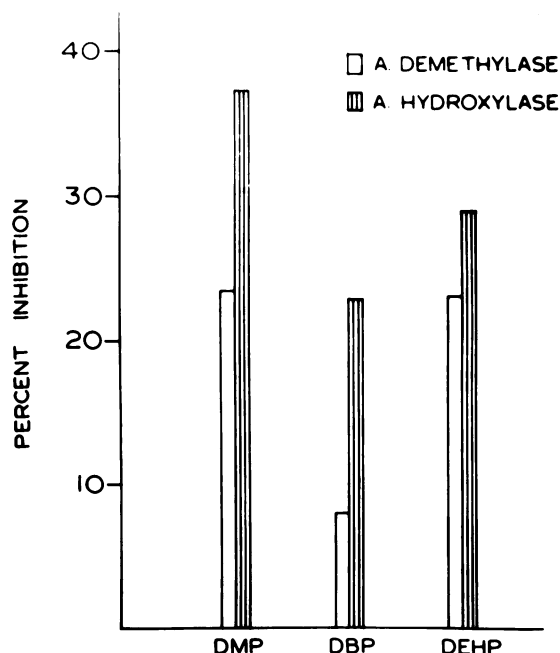


FIGURE 6. Effect of di(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP) and dibutyl phthalate (DBP) on aminopyrine *N*-demethylase (A. demethylase) and aniline hydroxylase (A. hydroxylase) activity of rat liver. Adult male albino rats were given DEHP, DMP, and DBP (5.0, 3.6 and 3.05 ml/kg, respectively) by intraperitoneal route, and enzyme activities were measured 18 hr after the treatment.

Conclusions

DEHP at high concentrations can cause functional hepatic damage, as reflected by morphological changes, and alterations in the activity of energy-linked enzymes and metabolism of lipids and carbohydrates. Phthalates can interact with other xenobiotics and such interactions occur during the pharmacokinetic phase. These observations are of significance, as man may be exposed to low levels of phthalates for prolonged periods which can cause altered drug-xenobiotic reactions. Effects of DEHP are dose- and route-dependent and are also influenced by strain and species of the test animal. At present, information on the biological effects of phthalates in higher animals is very limited.

REFERENCES

1. Thomas, J. A., Darby, T. D., Wallini, R. F., Garvin, P. J., and Martis, L. A review of biological effects of di(2-ethylhexyl) phthalate. *Toxicol. Appl. Pharmacol.* 45: 1-27 (1978).
2. Tomita, I., Nakamura, Y., and Yagi, Y. Phthalic acid esters in various food stuffs and biological materials. *Ecotoxicol. Environ. Safety* 1: 275-287 (1979).
3. Lawrence, W. H., and Tuell, S. F. Phthalate esters: the question of safety—an update. *Clin. Toxicol.* 15: 447-446 (1979).
4. Carpenter, D., Weil, C. S., and Smyth, S. F., Jr. Chronic oral toxicity of di(2-ethylhexyl) phthalate for rats, guinea pigs and dogs. *Arch. Ind. Hyg.* 8: 219-226 (1953).
5. Nikoronow, M., Mazur, H., and Peikacz, H. Effect of orally administered plasticizers and polyvinyl chloride stabilizers in the rat. *Toxicol. Appl. Pharmacol.* 26: 253-259 (1973).
6. Daniel, J. W., and Bratt, H. The absorption, metabolism and tissue distribution of di(2-ethylhexyl) phthalate in rats. *Toxicology* 2: 51-65 (1974).
7. Lake, B. G., Gangolli, S. D., Grasso, P., and Lloyd, A. G. Studies on hepatic effects of orally administered di(2-ethylhexyl) phthalate in the rat. *Toxicol. Appl. Pharmacol.* 32: 355-357 (1975).
8. Gray, T. J. B., Butterworth, K. R., Gaunt, I. F., Grasso, P., and Gangolli, S. D. Short-term toxicity study of di(2-ethylhexyl) phthalate in rats. *Food Cosmet. Toxicol.* 15: 389-399 (1977).
9. Srivastava, S. P., Agarwal, D.K., Mushtaq, M., and Seth, P. K. Effect of di(2-ethylhexyl) phthalate (DEHP) on chemical constituents and enzymatic activity of rat liver. *Toxicology* 11: 271-275 (1978).
10. Yamada, A. Toxicity studies on plasticizers. *J. Food Hyg. Soc. Japan* 15: 147 (1974).
11. Omori, Y. Recent progress in safety evaluation studies on plasticizers and plastics and their controlled uses in Japan. *Environ. Health Perspect.* 17: 203-209 (1976).
12. Lake, B. G., Branton, P. G., Gangolli, S. D., Butterworth, K. R., and Grasso, P. Studies on hepatic effects of orally administered di(2-ethylhexyl) phthalate in the ferret. *Toxicology* 6: 341-356 (1976).
13. Susumu, O. T. A., Hirotsuger, M. Y. U., and Thoshiyuki, K. Osakafuritsu Koshu Eisei Kenkyusho Kenkyu. *Hokoku Koshu Eiseiten* 15: 129 (1977).
14. Jacobson, M. S., Kevy, S. V., and Grand, R. J. Effects of a plasticizer leached from polyvinyl chloride on subhuman primate consequence of chronic transfusion therapy. *J. Lab. Clin. Med.* 89: 1066-1079 (1977).

15. Srivastava, S. P., Seth, P. K., and Agarwal, D. K. Biochemical effects of di(2-ethylhexyl) phthalate. *Environ. Physiol. Biochem.* 5: 178-183 (1975).
16. Ohyama, T. Effects of phthalate esters on respiration of rat liver mitochondria. *J. Biochem.* 79: 153-158 (1976).
17. Takahashi, T. Biochemical studies on phthalic acid esters II. Effects of phthalic acid esters on mitochondrial respiration of rat liver. *Biochem. Pharmacol.* 26: 19-24 (1977).
18. Inoye, B., Ogino, Y., Ishide, T., Ogata, M., and Utsumi, K. Effects of phthalate esters on mitochondrial oxidative phosphorylation in the rat. *Toxicol. Appl. Pharmacol.* 43: 189-198 (1978).
19. Srivastava, S. P., Agarwal, D. K., and Seth, P. K. Effects of di(2-ethylhexyl) phthalate on activity of succinic dehydrogenase and adenosine triphosphatase of some vital organs of rat. *Toxicology* 7: 163-168 (1977).
20. Seth, P. K., Srivastava, S. P., Agarwal, D. K., and Chandra, S. V. Effect of di(2-ethylhexyl) phthalate (DEHP) on rat gonads. *Environ. Res.* 12: 131-138 (1976).
21. Albro, P. W., Thomas, R., and Fishbein, L. Metabolism of diethylhexyl phthalate by rats. Isolation and characterization of urinary metabolites. *J. Chromatog.* 76: 321-330 (1973).
22. Williams, D. T., and Blenchfield, B. J. Retention, excretion and metabolism of di(2-ethylhexyl) phthalate administered orally to the rat. *Bull. Environ. Contam. Toxicol.* 11: 371-378 (1974).
23. Tanaka, A., Adachi, T., Takahashi, T., and Yamaha, T. Biochemical studies on phthalic esters. I. Elimination, distribution and metabolism of di(2-ethylhexyl) phthalate in rats. *Toxicology* 4: 253-264 (1975).
24. Sakurai, T., Miyazawa, S., and Hashimoto, T. Effects of di(2-ethylhexyl) phthalate administration on carbohydrate and fatty acid metabolism in rat liver. *J. Biochem.* 83: 313-320 (1978).
25. Mushtaq, M., Srivastava, S. P., and Seth, P. K. Effect of di(2-ethylhexyl) phthalate on glycogen metabolism in rat liver. *Toxicology* 16: 153-161 (1980).
26. Nazir, D. J., Alcaraz, A. P., Bierl, B. A., Beroza, M., and Nair, P. O. Isolation, identification and specific localization of di-2-ethylhexyl phthalate in bovine heart muscle mitochondria. *Biochemistry* 10: 4228-4232 (1971).
27. Stein, M. S., Cassi, P. I., and Nair, P. P. Influence of dietary fat and di-2-ethylhexyl phthalate on tissue lipids in rats. *J. Nutr.* 104: 187-191 (1974).
28. Reddy, J. K., Mody, D. E., Azarnoff, D. L., and Rao, M. S. Di(2-ethylhexyl) phthalate: an industrial plasticizer induces hypolipidemia and enhances hepatic catalase and carnitine acetyltransferase activities in rats and mice. *Life Sci.* 18: 941-945 (1976).
29. Osumi, T., and Hashimoto, T. Enhancement of fatty acyl-CoA oxidizing activity in rat peroxisomes by di(2-ethylhexyl) phthalate. *J. Biochem.* 83: 1361-1365 (1978).
30. Bell, F. P., and Nazir, D. J. Effect of di-2-ethylhexyl phthalate on lipid biosynthesis in selected tissues from rat *in vitro*. *Lipids* 11: 216-221 (1976).
31. Bell, F. P., Patt, C. S., Brundage, B., Gillies, P. J., and Phillips, W. G. Studies on lipid biosynthesis and cholesterol content of liver and serum lipoproteins in rats fed various phthalate esters. *Lipids* 13: 66-74 (1977).
32. Yangita, T., Kobayashi, K., and Enomoto, N. Accumulation of hepatic phospholipids in rats fed di-2-ethylhexyl phthalate. *Biochem. Pharmacol.* 27: 2283-2281 (1978).
33. Yangita, T., Kuzuhara, S., Enomoto, N., Shimada, T., and Sugano, M. Effects of di(2-ethylhexyl) phthalate on the content and composition of hepatic mitochondrial and microsomal phospholipids in the rat. *Biochem. Pharmacol.* 28: 3115-3121 (1979).
34. Pillai, K. S. R., and Seth, P. K. Influence of low protein diet on the toxicity of di(2-ethylhexyl) phthalate. *Ind. J. Biochem. Biophys.* 16 (Suppl.) Abstr. No. 243 (1978).
35. Calley, D., Autian, J., and Guess, W. L. Toxicology of a series of phthalate esters. *J. Pharm. Sci.* 55: 158-162 (1966).
36. Rubin, R. J., and Jaeger, R. J. Some pharmacologic and toxicologic effects of di(2-ethylhexyl) phthalate and other plasticizers. *Environ. Health Perspect.* 3: 53-59 (1973).
37. Seth, P. K., Srivastava, S. P., Agarwal, D. K., and Mushtaq, M. Interaction of di(2-ethylhexyl) phthalate with pentobarbitone and methaqualone. *Bull. Environ. Contam. Toxicol.* 6: 727-732 (1977).
38. Lawrence, W. H., Malik, M., Turner, J. E., Singh, A. R., and Autian, J. A. A toxicological investigation of some acute, short-term and chronic effects of administering di(2-ethylhexyl) phthalate and other phthalates. *Environ. Res.* 9: 1-11 (1975).
39. Srivastava, S. P., Agarwal, D. K., Mushtaq, M., and Seth, P. K. Interaction of di(2-ethylhexyl) phthalate with parathion in rats. *Chemosphere* 5: 177-181 (1977).
40. Seth, P. K., Srivastava, S. P., Mushtaq, M., Agarwal, D. K., and Chandra, S. V. Effect of di(2-ethylhexyl) phthalate on rat liver injured by chronic carbon tetrachloride treatment. *Acta Pharmacol. Toxicol.* 44: 161-167 (1979).
41. Agarwal, D. K., and Seth, P. K. Interaction of di(2-ethylhexyl) phthalate with ethanol metabolism in mice. Abstracts All India Symposium of Xenobiotics Metabolism. Aurangabad, India.
42. Singh, A. R., Lawrence, W. H., and Autian, J. Teratogenicity of phthalate esters in rats. *J. Pharm. Sci.* 61: 51-55 (1972).
43. Agarwal, D. K., et al. Unpublished data.
44. Agarwal, D. K., and Seth, P. K. Unpublished data.
45. Lake, B. G., Philips, J. C., Linnell, J. C., and Gangolli, S. D. The *in vitro* hydrolysis of some phthalate esters by hepatic and intestinal preparations from various species. *Toxicol. Appl. Pharmacol.* 39: 239-248 (1977).
46. Carter, J. E., Roll, D. B., and Peterson, R. V. The *in vitro* hydrolysis of di(2-ethylhexyl) phthalate by rat tissues. *Drug Metab. Disp.* 2: 341-344 (1974).
47. Peck, C. C., Odom, D. G., Friedman, H. I., Albro, P. W., Hass, J. R., Brady, J. T., and Jess, D. A. Di-2-ethylhexyl phthalate (DEHP) and mono-2-ethylhexyl phthalate (MEHP) accumulation in whole blood and red cell concentrates. *Transfusion* 19: 137-146 (1979).
48. Kasuya, M. Toxicity of phthalate esters to nervous tissue in cell culture. *Bull. Environ. Contam. Toxicol.* 12: 167-172 (1974).